Special Article

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Comparison of antimicrobial susceptibility testing practices in 20 clinical microbiology laboratories in India

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Introduction

The world is facing a unique crisis with regard to the rise of antimicrobial resistance (AMR). Clinical microbiology laboratories have a critical role in the effort to combat antibiotic resistance. However, antimicrobial susceptibility testing (AST) practices are not uniform in India because of the diversity of the guidelines available (Clinical Laboratory Standards Institute Manual [CLSI], EUCAST), variation in institutional/individual preferences, differences in training, expertise, infrastructure and technology available. Understanding this variation of practices is important for clinicians, microbiologists, policymakers and researchers so that the strength and limitation of the data generated could be appreciated. Previous studies from the USA including the CDC has reported (from 116 episodes of bacteraemia from 14 participating hospitals in the US) that AST errors or reporting errors were found in 16% and reporting of AST results to be inappropriate for 33%.[1] Such aberrations are not unlikely in the Indian context.

This special article was conceptualised to address the role of the clinical microbiologist in tackling antibiotic resistance by accurate testing, interpretation and reporting of antibiotic susceptibility tests. The questionnaire survey conducted across several hospital-based microbiology laboratories in India [Figure 1] aimed to cover the entire process by which a microbiology report is generated leading to the clinician choosing an antibiotic for a certain infection. The questionnaire survey was conducted over emails, which were sent to departmental heads of microbiology. Centres for sending emails were chosen based on the familiarity of the members of the Academy of the Clinical Microbiologists, India, to the editorial team. Altogether emails were sent to those institutions/individuals likely to respond to the survey. Twenty centres participated in the questionnaire survey (16 from Kerala and one each from Karnataka, Puducherry, Maharashtra and West Bengal). Names of the individual centres were coded and left anonymous to maintain confidentiality. Numbering of the centres was done based on a descending order of total number of isolates (i.e., centres having the maximum number of isolates were given the first

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number and the centre with the least number of isolates was given the 20th number). Analysis was done based on recommendations given in CLSI 2016 guidelines.[2] All centres were large, multispeciality hospitals, and the average number of organisms isolated was 4206. The ratio of Gram-negatives to Gram-positives was 70:30 in most centres except centre number 14, 17 and 20 where close to 50% of the isolates were Gram-positives. Non-fermenters formed a sizable population of 17%–20% of the total isolates except in two centres, namely numbers 9 and 10.

Table 1 shows the first set of data pertaining to the total number of organisms. This has been arranged according to the total number of isolates in the year 2016 in descending order and the centres have thus been numbered from 1 (highest) to 20 (lowest).

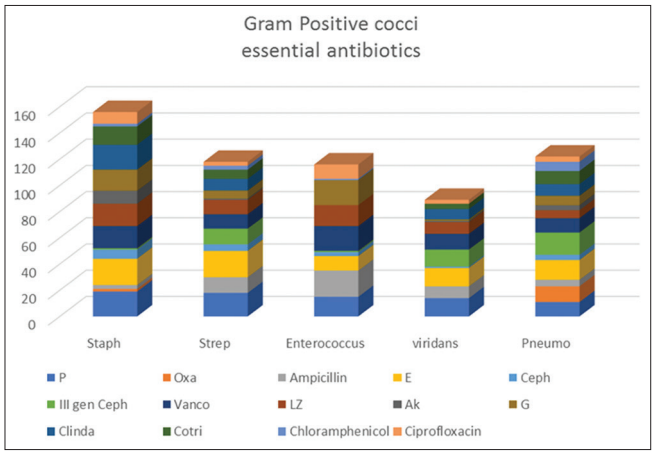

Figure 1: The choice of primary antibiotics for Gram-positive cocci in various microbiology laboratories

The first set of questions was regarding the antibiotics tested for Gram-positive bacteria.

Despite low sensitivity to Penicillin for most staphylococci testing for Penicillin is still mandatory in all laboratories. Oxacillin has replaced Cefoxitin in all laboratories. First-generation cephalosporin (such as Cefazolin) is a good alternative to Cloxacillin in clinical settings against *Staphylococcus aureus*; however, it is not included in the testing panel for staphylococci in CLSI 2016 guidelines.[2] It was found that seven laboratories tested for first-generation cephalosporins. Testing separately for first generation cephalosporin can be misleading because it may show large zones even in Methicillin Resistant *S. aureus* (MRSA) but has to be reported as resistant.

Daptomycin was tested only in one laboratory. Pristinamycin was also tested only in one laboratory (although not mentioned at all in the CLSI 2016 guidelines).[2] One laboratory tested third-generation cephalosporin against enterococci which are inherently resistant to all cephalosporins. Ciprofloxacin may be used for Gram-positive urinary isolates (such as Enterococcus), but it is used to test *Streptococcus pneumoniae (S.pneumoniae)* in four centres and streptococci including viridans streptococci in three centres. It is an

Table 1: Summary of bacterial isolates from twenty hospital-based microbiology laboratories in India

Lab serial number	Total isolates	GPC (%)	Enterobacteriaceae (%)	Non-fermenters (%)	Summation	Other isolates
1	10,080	3939 (39.1)	3764 (37.3)	2131 (21.1)	97.6	2.4
2	9417	1896 (20.1)	5194 (55.2)	2088 (22.2)	97.5	2.5
3	9121	2542 (27.9)	4901 (53.7)	1678 (18.4)	100.0	0.0
4	5750	1237 (21.5)	3333 (58)	1180 (20.5)	100.0	0.0
5	5430	1718 (31.6)	2374 (43.7)	975 (18)	93.3	6.7
6	5379	1871 (34.8)	2529 (47)	981 (18.2)	100.0	0.0
7	4590	1275 (27.8)	2034 (44.3)	985 (21.5)	93.6	6.4
8	4458	1504 (33.7)	1999 (44.8)	948 (21.3)	99.8	0.2
9	4323	1554 (35.9)	2496 (57.7)	267 (6.18)	99.9	0.1
10	3875	1325 (34.2)	2520 (65)	30 (0.77)	100.0	0.0
11	3404	695 (20.4)	2017 (59.3)	686 (20.2)	99.8	0.2
12	3028	609 (20.1)	1543 (51)	578 (19.1)	90.2	9.8
13	2895	662 (22.9)	1521 (52.5)	712 (24.6)	100.0	0.0
14	2540	1230 (48.4)	860 (33.9)	450 (17.7)	100.0	0.0
15	2298	592 (25.8)	1234 (53.7)	472 (20.5)	100.0	0.0
16	1942	839 (43.2)	819 (42.2)	242 (12.5)	97.8	2.2
17	1884	916 (48.6)	685 (36.4)	283 (15)	100.0	0.0
18	1873	731 (39)	814 (43.5)	328 (17.5)	100.0	0.0
19	1206	420 (34.8)	576 (47.8)	210 (17.4)	100.0	0.0
20	637	333 (52.3)	231 (36.3)	71 (11.1)	99.7	0.3
Median	3639.5	1233.5 (34.0)	2008 (47.4)	632 (18.3)	99.7	0.3
Average	4206.5					

GPC: Gram-positive cocci

antibiotic that could be reserved for salmonella and urinary infections but misuse originates because of anomalous testing in the microbiology laboratory.

Methicillin resistance was tested by Cefoxitin disc method in all 20 centres. MRSA is a challenge due to the lesser number of antibiotic choices for treatment and its propensity for spread. The number of MRSA expressed as a percentage of the total number of organisms isolated in the centre gives an idea of the size of the threat in that particular centre. This can be seen in Figure 2.

In Figure 2, it can be seen that Clindamycin resistance is also tested for and detected in most laboratories. It can be innate when it is easily detected by a small zone on the sensitivity plate. However, it is also inducible, and this is detected by the Erythromycin disc which if kept near the Clindamycin disc shows a straight line when the two zones merge, which appears like a capital D. There are four centres that do not test for inducible Clindamycin resistance. This can lead to false reporting of sensitivity where, if Clindamycin is used, rapid development of resistance may result.[2]

While manual methods were followed everywhere, five centres do have access to VITEK 2 and one centre uses Phoenix. Oxacillin agar screening for MRSA was done by one centre.

Rare Resistance Patterns

Vancomycin resistance in staphylococci (VRSA) is quite rare (only two centres reported it). VRSA is confirmed by testing for minimum inhibitory concentration (MIC) (isolates for which Vancomycin MICs are ≥16 µg/mL are classified as Vancomycin resistant) and by molecular methods.[3] In enterococci, Vancomycin resistance was reported from nine centres. The highest rate was 1.1% in centre 12. Resistance to Penicillin is an emerging threat in *S. pneumoniae*.

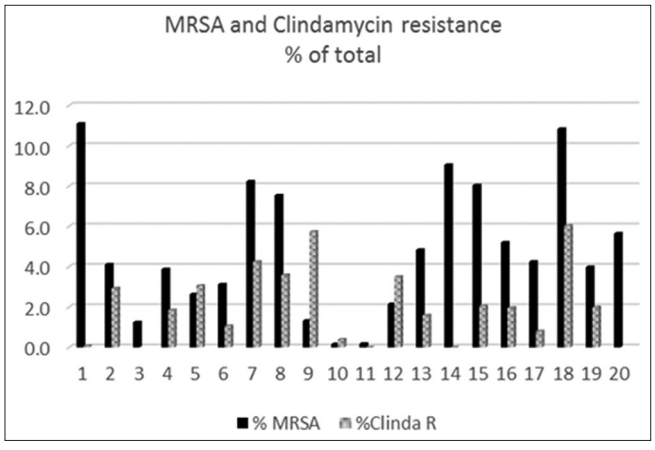

Figure 2: Methicillin-resistant *Staphylococcus aureus* and Clindamycin resistance

This leaves only the third-generation cephalosporins, carbapenems, glycopeptides (e.g., Vancomycin) and macrolides for therapy. Centre number 4 reported the highest number with 0.2% of the total isolates being Penicillin-resistant *S. pneumoniae*. There were six other centres which reported resistant *S. pneumoniae*.

Other Antibiotics Used for Gram-positives

The above were only the primary antibiotics used to test Gram-positives. The other antibiotics used regularly included those shown in Table 2.

There was only one centre that used Gram-negative panels fully for Gram-positives. The treating doctors in that centre perhaps liked to continue the antibiotic that the patient was already on and asked the laboratory to test for those too. This leads to antibiotic misuse.

Enterococci and staphylococci are known to cause urinary infections, and so urinary antibiotics are tested for urinary isolates in a few centres.

Clinical Laboratory Standards Institute Manual and Testing for Gram-positives: Interpretative Reading

Staphylococci

- Penicillin sensitive indicated sensitivity to Ampicillin and Amoxicillin
- Oxacillin sensitive (by Cefoxitin) indicated sensitivity to first-generation cephalosporins and other cephalosporins with anti-Gram-positive activity, combinations such as Ampicillin-Sulbactam and Amoxicillin-clavulanic acid and all carbapenems, which therefore, do not need to be tested separately
- Oxacillin-resistant staphylococci are likewise resistant to all above agents including Penicillin, cephalosporins and carbapenems, which need not be tested separately
- Ceftaroline is a new fifth-generation cephalosporin which can now be tested in MRSA, but none of the centres has reported testing for it
- For Vancomycin and Daptomycin, only MIC is reliable and those with any of the automated systems can test these and need to be verified preferably by another MIC based method
- Aminoglycosides can be tested but may be used only in combination
- If tetracyclines are used in treatment, Doxycycline and Tetracycline should be tested separately; however, Erythromycin can be surrogate for Azithromycin and Clarithromycin
- In case of quinolones, CLSI gives a cautionary note that resistance develops very fast. It is a well-known fact that Ciprofloxacin can select out MRSA from a heterogeneous population. It is thus best avoided.

Table 2: Extended set of antibiotics used for Gram-positive cocci in different centres

Antibiotic	Staphylococcus	Streptococcus	Enterococcus	*Viridans streptococci*	Pneumococci	Comments
Teicoplanin	8	1	7	2	0	
Rifampicin	3	1	0	0	0	
Tetracycline	7	2	4	1	4	
Doxycycline	3	2	0	2	2	
Azithromycin	0	0	0	2	4	
Cefepime	0	0	0	2	2	
Levofloxacin	0	7	3	2	7	
Ofloxacin	0	1	0	0	1	
Moxifloxacin	0	0	0	0	1	
Co-amoxiclav	3	3	0	2	2	No CLSI criteria for beta haemolytic Streptococci, *Viridans Streptococci* and staphylococci
Aztreonam	0	0	0	1	1	Inappropriate selection
Cefoperazone-Sulbactam	0	0	0	1	1	No CLSI criteria
Piperacillin-tazobactam	0	0	0	1	1	No CLSI criteria
Colistin	0	0	0	1	1	Inappropriate selection
Meropenem	0	0	1	1	1	No CLSI criteria for enterococcus and *Viridans streptococci*
Imipenem	0	0	1	1	1	No CLSI criteria for enterococcus and *Viridans Streptococci*
Tigecycline	1	0	1	0	0	No CLSI criteria
Fosfomycin	0	0	1	0	0	
Nitrofurantoin	4	1	10	0	0	
Norfloxacin	3	1	5	0	0	

CLSI: Clinical Laboratory Standards Institute

Enterococci

- Ampicillin/Amoxicillin are the drugs of choice in susceptible strains. Penicillin must be tested separately as those sensitive to Ampicillin may not be sensitive to Penicillin. However, if Penicillin sensitive, it is sensitive to Ampicillin
- Vancomycin may be tested by disc diffusion, but any haze within the zone should be tested by MIC
- Cephalosporins, Clindamycin and Co-trimoxazole may appear sensitive *in vitro* but are not effective clinically; hence, it is not advisable to test enterococci for these
- Aminoglycosides that appear resistant may actually be useful when combined with a Beta-lactam agent. Hence, testing for high-level aminoglycoside resistance (HLAR) is helpful. Most of the centres are aware of this and use 120 µg discs for this. E-test strip is also useful to get MIC in these cases
- Fosfomycin is a drug that can be used to test *Enterococcus faecalis*. Best method is agar dilution. A 200 µg disc with glucose-6-phosphate incorporated is available
- Other antibiotics such as Erythromycin, Tetracycline, quinolones, Rifampicin and Chloramphenicol may be tested in highly resistant strains
- Cremaschi *et al.* recommend testing Linezolid in biliary tract infections, as it has excellent pharmacokinetics in such infections.[4]

Streptococcus pneumoniae (Pneumococci)

- Pneumococci are the most difficult to interpret by disc diffusion. Oxacillin disc may help in predicting Penicillin susceptibility; if resistant to Oxacillin, Penicillin MIC needs to be done, especially in meningitis. However, for all generations of Cephalosporins, MIC is mandatory (except Ceftaroline, a non-meningitis indication)
- Vancomycin can be tested by disc diffusion.

Streptococci

- In case of *Streptococcus pyogenes*, Penicillin is tested by disc diffusion, and it is a surrogate marker for antibiotics with the beta-lactam ring and anti-Gram-positive activity, which includes all penicillins, cephalosporins and carbapenems. If found resistant to Penicillin, its identification should be confirmed and then sent to a referral laboratory for confirmation of sensitivity testing results
- Viridans streptococci are the main agents of infective endocarditis. Hence, in such isolates, Penicillin testing by E-test strips is required. A high MIC, though sensitive by ordinary disc diffusion, may merit higher doses of the drug.

In all streptococci, D-test for Clindamycin is recommended. Aminoglycosides are reported as sensitive when they are HLAR negative. However, they cannot be used for monotherapy.

Antibiotics Tested for Gram-negative Bacteria

Gentamicin and quinolones were tested uniformly at all centres for both enterobacteriaceae and non-fermenters [Figure 3]. Ceftazidime was tested only for non-fermenters in some centres. For enterobacteriaceae, all except two centres still tested for Ampicillin, whereas two centres tested for Ampicillin in non-fermenters as well. Third-generation cephalosporins, quinolones, carbapenems, aminoglycosides and Co-trimoxazole were tested for both. However, Cefoperazone-Sulbactam is more used in enterobacteriaceae whereas Piperacillin-tazobactam is more popular in non-fermenters. Other combinations used are Ceftazidime-clavulanic acid, Ticarcillin-clavulanic acid and Cefepime-tazobactam.

Polymyxin B is used in three centres only, whereas Colistin is used in 14 centres for enterobacteriaceae.

CLSI does not recommend routine testing to determine the mechanism of resistance. All large hospitals find it necessary to determine the mechanism of resistance for epidemiological purpose. Mechanism also helps in interpretative reading.[5] Mechanism of resistance may depend on the prescribing practices in particular centres and infection control practices. Data of this kind help in planning methods to reduce resistance. Phenotypic methods are rather subjective, difficult to interpret without experience and inconclusive at times. Molecular methods are necessary, and a few centres may emerge as pioneers in this field in the days to come.

There are six centres that do not look at mechanism of resistance at all. Amongst the other centres, eight centres look for extended spectrum beta-lactamases (ESBLs) by phenotypic methods. AmpC is detected at seven centres by phenotypic methods. Only four centres go for the modified Hodge test to detect carbapenemase. Metallo-beta-lactamase is detected by phenotypic test using Imipenem-EDTA to chelate the metallic zinc molecule in three centres. None of the centres use these data while reporting on a routine basis. No centre reported the use of molecular methods for confirmation.

Using evidence-based antibiotic susceptibility testing guidelines (such as CLSI or EUCAST) enables accurate reporting. Automated systems such as VITEK are useful in ensuring greater accuracy and reproducibility. There were six centres that give report according to VITEK 2.

AmpC beta-lactamase confers resistance to beta-lactam-lactamase inhibitor combinations as well. These are used extensively when resistance to third-generation cephalosporins is detected. Genera such as *Escherichia coli*, Klebsiella and Enterobacter have now acquired the plasmids for this and though they may appear sensitive initially, may turn resistant due to inducible AmpC.[6] Report about this mechanism of resistance and its clinical implication must be communicated to clinicians.

Amongst unusual resistance detected, Colistin resistance seems to have emerged in four centres. Figure 4 shows the rate of resistance to Colistin and Tigecycline. There are two centres that do not test for Colistin. Testing for Colistin by disc diffusion can tell if there is absolute resistance. However, to detect doubtful cases, MIC is essential. Either E-test or automated systems such as VITEK 2 may be used for this. We suggest confirming all possible Colistin-resistant isolates by multiple methods, for treatment and infection control implications.

Table 3 shows the testing aberrations observed in several centres that may lead to inappropriate antibiotic use and lack of *in vivo* response to treatment of an antibiotic reported as susceptible. This will cause a lack of confidence in the laboratory.

Reporting Culture and Sensitivity Results

Testing, reading, interpreting and reporting are four distinct functions done in a microbiology laboratory, whether it is bacteriology, mycology, virology or parasitology. The third part of the questionnaire dealt

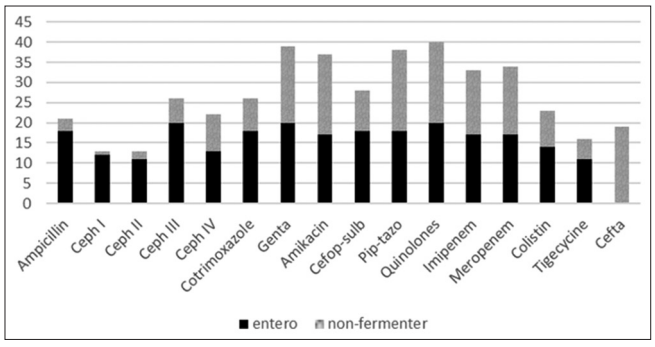

Figure 3: Major antibiotics tested in Gram-negative bacteria

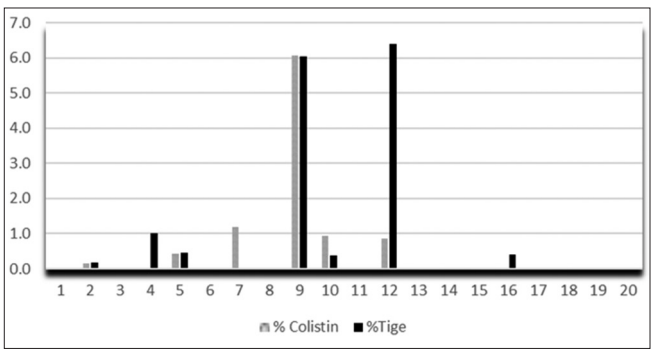

Figure 4: Resistance to Colistin and Tigecycline

Table 3: Testing aberrations observed in some laboratories through the questionnaire survey on antibiotic susceptibility testing practices

Third-generation Cephalosporin testing for enterococci
Ampicillin and Co-trimoxazole susceptibility testing for all non-fermentative Gram-negative bacilli
Cefoperazone-sulbactam susceptibility testing without CLSI interpretative criteria
Ciprofloxacin testing for *Streptococcus pneumonia*, alpha haemolytic streptococci
Not testing for inducible clindamycin resistance in Staphylococci
Pristinamycin testing: No interpretative criteria exist in CLSI 2016
Testing Gram-negative antibiotic panels (e.g., Aztreonam and Colistin) for GPC

CLSI: Clinical Laboratory Standards Institute, GPC: Gram-positive cocci

with the approach to communication with the primary treating physician/departments who has the primary responsibility in the clinical care of the patient.

Restrictive reporting is one of the components of antibiotic stewardship. There can be two approaches to it. The laboratory can reduce the number of antibiotics tested and report only those tested. The second approach is testing all the available antibiotics, perhaps keeping a different panel for various categories of organisms and reporting only a relevant few.

For getting an idea of the policy of the laboratories, three questions were asked - (a) whether restrictive reporting was practised, if so in what way, (b) whether drug of choice was indicated in the report and (c) whether distinction was made between colonisation and infection while giving the report. These questions indicate the work done by clinical microbiologists behind each report that goes out.

We observed that 11 out of 20 centres have given an affirmative to all three questions. The CLSI has divided the antibiotics for each organism into groups A, B, C, U and O. A denotes the primary antibiotics, B the primary to be selectively reported, C for supplemental testing and selective reporting. U is for urinary isolates and Group O ('other') includes antimicrobial agents that have a clinical indication for the organism group but are generally not candidates for routine testing and reporting. Out of 20, 16 centres report according to these principles.

Drug of choice is the drug that is best for a certain species of bacteria, for example, Penicillin for *S. pyogenes*. Out of 20 centres, 12 suggest the best antibiotic to be given for the infection. This can be taken up by other centres also since it leaves the clinician free to concentrate on the primary treatment that his speciality is giving, such as cardiovascular or respiratory drugs. If there are interactions that may affect the patient, the clinician will be encouraged to discuss this with the clinical microbiologist, paving the way for laboratory guidance of therapy.

For distinguishing infection and colonisation, most laboratories felt the need to leave this to the clinician concerned. However, out of 20 centres, 17 have mechanisms in place to distinguish coloniser from pathogen. This may imply that clinical microbiologists are increasingly connecting with the patient rather than with the specimen alone.

The current article based on the results of the questionnaire survey of 20 hospital-based clinical microbiology laboratories from India demonstrate that although there is a significant convergence of laboratory standards and practices, there are also major deviations noted in a few (as compared to international and national guidelines). It is hoped that with greater awareness generated amongst clinicians and microbiologists, uniform standards may be developed in the foreseeable future. The Standard Operating Procedure in Bacteriology of the Indian Council of Medical Research is an effort in this direction.[7]

It may be concluded that clinical microbiologists are increasingly adapting and evolving their practice to keep up with the ever-changing world of microbes, technology and their interactions. This special article highlights the fact that there are several components to a microbiology report that must be carefully thought out, planned and implemented in the practice of clinical microbiology so that patients and treating physicians are benefited.

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Conflicts of interest

There are no conflicts of interest.

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